GUIDANCE[1]

CARBIDOPA AND LEVODOPA TABLETS

IN VIVO BIOEQUIVALENCE

AND *IN VITRO* DISSOLUTION

I. INTRODUCTION

A. Pharmacology

Levodopa is used in the treatment of Parkinson's disease. The symptoms of parkinsonian syndrome are related to depletion of dopamine in the corpus striatum. Following oral administration, about 95% of levodopa is rapidly decarboxylated to dopamine which does not cross the blood-brain barrier. Because less than 1% of absorbed levodopa penetrates the CNS and only a small amount enters the brain, large doses of levodopa need to be administered. This allows enough accumulation of levodopa in the brain to raise the dopamine concentration by enzymatic decarboxylation (1-4). Carbidopa is a peripheral decarboxylase inhibitor which inhibits decarboxylation of levodopa to dopamine. Thus, more levodopa is available for transport to the brain (1-4).

Carbidopa/levodopa is contraindicated in patients receiving monoamine oxidase inhibitors, in patients

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[1]This statement, prepared by the Division of Bioequivalence in the Office of Generic Drugs, is an informal communication under 21 CFR 10.90(b)(9) that represents the best judgment of the Division at this time. This statement does not necessarily represent the formal position of the Center for Drug Evaluation and Research, Food and Drug Administration, and does not bind or otherwise obligate the Center for Drug Evaluation and Research, Food and Drug Administration, to the views expressed. For further information about this guidance, contact the Division of Bioequivalence, Office of Generic Drugs, 7500 Standish Place, Metro Park North, Rockville, MD 20855 (Phone: 301-295-8290; Fax: 301-295-8183).

with known hypersensitivity to this drug, and in narrow angle glaucoma. The drug should not be used in patients with suspicious, undiagnosed skin lesions or a history of melanoma (1).

The most common serious adverse reactions occurring with Carbidopa/levodopa are choreiform, dystonic, and other involuntary movements. Other serious adverse reactions are mental changes, including paranoid ideation and psychotic episodes, depression with or without development of suicidal tendencies, and dementia. Convulsions also have occurred. A common but less serious effect is nausea (1).

Levodopa is commercially available alone in three strengths: 100 mg, 250 mg and 500 mg tablets. Carbidopa and levodopa combination tablets are commercially available in three strengths: 10 mg/100 mg, 25 mg/100 mg and 25 mg/250 mg as Sinemet ^{R} (Merck Sharp & Dohme). The optimum daily dosage must be determined by careful titration in each patient. Dosage is best initiated with one 25 mg/100 mg tablet three times a day. Dosage may be increased by one tablet every day or every other day, as necessary, until a dosage of eight 25 mg/100 mg tablets a day is reached (1).

B. Chemistry

Levodopa is the levorotatory isomer of dihydroxyphenyl-alanine, (-)-3-(3,4-dihydroxyphenyl)-L-alanine, and the metabolic precursor of dopamine. Levodopa occurs as a white to off-white, odorless, crystalline powder and is slightly soluble in water and insoluble in alcohol.

The chemical name of carbidopa is S-alpha-hydrazino-3,4-dihydroxy-alpha-methylbenzenepropanoic acid monohydrate. Carbidopa occurs as a white to creamy white, odorless or practically odorless powder and is slightly soluble in water and practically insoluble in alcohol.

Levodopa and carbidopa are rapidly oxidized and darken in the presence of moisture. Commercially available preparations containing levodopa and/or carbidopa should be protected from exposure to light, moisture, and excessive heat (3). The chemical structures of levodopa and carbidopa are shown in the following figure:

C. Pharmacokinetics

Levodopa is rapidly and almost entirely absorbed from the GI tract by an active transport system for aromatic amino acids. Concentration of levodopa in plasma after an oral dose usually peaks within two hours. The plasma half-life is short, approximately 1 to 3 hours. Studies show that levodopa absorption is faster under fasting conditions. However, similar AUC's were observed under fasting and nonfasting conditions (5-9).

Levodopa is widely distributed into most body tissues and the total volume of distribution is about 1 L/kg body weight for elderly subjects (9). Most of the absorbed levodopa is decarboxylated to dopamine, small amounts of which are metabolized to norepinephrine and epinephrine. Dopamine is further metabolized to 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid and excreted in urine (2,6).

Nonlinear pharmacokinetics of levodopa has been observed with oral administration of levodopa alone. The nonlinearity is attributed to saturable first-pass metabolism during absorption (10). Levodopa bioavailability is lower in young volunteers than in elderly volunteers. This is attributed to age-related decreases in both gastric emptying and first-pass metabolic decarboxylation in the GI tract and also to reduced plasma clearance in elderly subjects (7-9).

Carbidopa is a decarboxylase inhibitor which inhibits decarboxylation of levodopa to dopamine. Upon oral administration of levodopa, dopamine is found widely distributed in most body tissues but is unable to penetrate the blood-brain barrier. Concurrent administration of carbidopa inhibits the peripheral decarboxylation of levodopa without affecting the metabolism of the drug within the CNS. Thus, more levodopa is available for transport to the brain (1-4). Although levodopa does not appear to enhance the absorption of carbidopa, carbidopa may enhance the absorption of levodopa by suppressing the metabolism of levodopa in the GI tract. Plasma levodopa concentrations are increased when carbidopa and levodopa are administered concomitantly, principally due to inhibition of the peripheral metabolism of levodopa by carbidopa (2,5,6).

About 40-70% of a carbidopa dose is absorbed following oral administration. The drug is not extensively metabolized (5,6). The plasma half-life of carbidopa is 1-2 hours (3).

II. **BIOEQUIVALENCE STUDIES**

A. Types of Studies Required

1. A randomized, single-dose, two-treatment, two-period, crossover study under fasting conditions with the 25 mg/250 mg strength generic test product compared to the reference product, Sinemet® 25 mg/250 mg tablet.

2. *In vitro* dissolution testing for the 25 mg/250 mg strength.

3. Waivers from bioequivalence study requirements may be granted for the 10 mg/100 mg and 25 mg/100 mg

strengths of carbidopa/levodopa tablets if the following conditions are met:

a. The sponsor has an approved bioequivalence study for the 25 mg/250 mg strength product as described above.

b. The 10 mg/100 mg and 25 mg/100 mg strength products are proportionally similar in their active and inactive ingredients to the 25 mg/250 mg strength.

c. The 10 mg/100 mg and 25 mg/100 mg strength products meet an established *in vitro* dissolution specification.

B. Fasting Study

Objective: The objective of this study is to compare the bioavailability of generic carbidopa and levodopa tablets, 25 mg/250 mg strength, (test product) with that of the reference product, Sinemet ® 25 mg/250 mg tablet (Merck Sharp & Dohme) under fasting conditions.

Design: The study design is a randomized, single dose, two treatment, two period, two sequence crossover with a washout period of at least seven days. Subjects should be randomly assigned to dosing sequences.

Facilities: The clinical and analytical sites for the study should be given along with the names, titles and the curriculum vitae of the medical, scientific and analytical directors. The starting and ending dates for each clinical study period should be stated. The study protocols should be approved by an institutional review board, and informed consent forms should be signed by all participants.

Subjects: A minimum of 30 subjects should be used. It is the sponsor's responsibility to use a sufficient number of subjects to ensure adequate statistical significance of results.

The subjects should be healthy male volunteers between 18-45 years of age and within 10% of the ideal weight for their height and body frame according to the Metropolitan Insurance Company Bulletin, 1983. All

subjects should be given a physical examination and appropriate laboratory tests 4 weeks prior to the initiation of the study. These should be repeated at the end of the study.

Exclusion Criteria: Subjects should be excluded from the study using the following criteria and other criteria deemed necessary by the sponsor:

1. History of serious systemic or organ disease including, but not limited to, cardiovascular, renal, hepatic, gastrointestinal, hematopoietic, or neurological disease, and asthma, tuberculosis, epilepsy, glaucoma, or mental illness.

2. Significant physical or organ abnormality.

3. History of past or recent alcohol or drug addiction or abuse.

4. History of or known hypersensitivity to levodopa or carbidopa.

5. Subjects with suspicious, undiagnosed skin lesions or a history of melanoma.

6. Exposure to known hepatic enzyme inducing or inhibiting agents such as reserpine, phenothiazine, phenobarbital and carbamazepine within 30 days prior to the study.

7. Use of any prescription drug product such as MAO inhibitors or any OTC drug product such as multivitamins containing pyridoxine within two weeks prior to the study.

8. Participation in an investigational drug study within 30 days prior to the study.

9. Blood donation within 30 days prior to the study.

10. Tobacco use in any form.

Procedures: After an overnight (at least 10 hours) fast, subjects should receive a single dose of the test product or the reference product with 240 ml of water:

Treatment A: Test product, 1 X 25 mg/250 mg carbidopa/levodopa tablets.

Treatment B: Reference product, 1 x 25 mg/250 mg Sinemet® Tablets (Merck Sharp & Dohme).

The test product should be from a production lot or from a lot produced under production conditions. The lot size of the test product should be equal to or more than 100,000. The lot numbers of both the test and reference products and the expiration date for the reference product should be stated. The potency of the reference product should not differ from that of the test product by more than ± 5%. The sponsor should include a statement of the composition of the test product.

The clinical staff administering the doses should verify that the dose was taken in all cases. After at least a one week washout period after the last blood sample, each subject should receive the alternative treatment.

Restrictions: Prior to and during each study phase, subjects will observe the following restrictions:

1. Water will be allowed *ad libitum* except for one hour before and after drug administration. Subjects should be kept well hydrated prior to and during each study phase, and the amount of fluid should be kept constant throughout each study period.

2. Subjects should be served standardized meals no less than 4 hours after drug administration. Only standardized meals and beverages at specified times will be allowed during the study.

3. No alcohol or xanthine-containing foods or beverages should be consumed for 48 hours prior to each study period and until after the last blood sample is collected.

4. Subjects will be confined to the clinical facility for 24 hours after each dosing. With permission of the Medical Director, subjects may then be released to return for the post-study tests.

Blood Sampling: Blood samples in a volume sufficient for sample analysis and anticoagulated as appropriate may be collected at 0 hr (pre-dose), and at 0.25, 0.5, 0.75, 1, 1.33, 1.66, 2, 2.5, 4, 6, 8, 10, 12, 16, and 24 hr post-dose. Samples should be centrifuged promptly and plasma separated and frozen at -20 °C until assay. For each subject, the sponsor should state the time elapsed between sample collection and sample assay. An explanation should be given for any missing samples.

Urine Samples: Urine samples should be collected over the time intervals of -2-0, 0-2, 2-4, 4-6, 6-8, 8-10, 10-12, and 12-24 hours post-dose. Urine samples will be assayed if needed.

Subject Monitoring: The physical examination and clinical laboratory tests should be repeated during the washout period and after completing the biostudy. Blood pressure and pulse rate should be monitored during the blood sampling periods.

Analytical Methods: For measurement of levodopa and carbidopa levels in the blood (or urine if needed), HPLC or another comparable analytical method should be selected (13). The method used should be described in detail and references, if any, should be cited. The method should include detailed calculation procedures for the assay results.

Due to the poor chemical stability of levodopa under exposure to light and air, care must be taken to prevent the degradation of levodopa during handling and storage of blood and urine samples. Freshly prepared standards should be used in the assay.

In general, the sponsor should select a method that will ensure specificity, accuracy, interday and intraday precision, linearity of standard curves and adequate sensitivity. Quality Control samples in the low, middle, and high ranges of the standard curve should be prepared (separate weighings for each control concentration) on the same day as the study samples are collected.

The sponsor should submit pre-study validation data with respect to assay accuracy, precision, recovery,

linearity, and lowest limit of quantitation. Stability of the samples under frozen conditions, at room temperature, and during freeze-thaw cycles, if appropriate, should be determined (14).

Pharmacokinetic Analysis: From plasma drug concentration-time data, the sponsor should obtain the following pharmacokinetic parameters for levodopa and carbidopa:

1. AUC_{0-t}, calculated by the trapezoidal rule, where t is the last measurable time point.

2. $AUC_{0-\infty}$, where $AUC_{0-\infty} = AUC_t + C_t/(\lambda_Z)$, C_t is the last measurable drug concentration and λ_Z is the terminal elimination rate constant.

3. The terminal phase elimination rate constant (λ_Z) is calculated using an appropriate pharmacokinetic method.

4. Peak drug concentration (C_{max}) and the time to peak drug concentration (T_{max}) are obtained directly from the data without interpolation.

Statistical Analysis: The sponsor should perform the following tests:

1. Analysis of variance (ANOVA) appropriate for a crossover design on the pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} using General Linear Models (GLM) procedure of SAS (11) or an equivalent program should be performed. The statistical model should include terms describing the effects attributable to sequence, subject(sequence), period, and treatment. The sequence effect should be tested using the between-subject main effect [subj(seq)] as an error term. All other main effects should be tested against the residual error (error mean square) from the ANOVA.

2. The ESTIMATE statement in SAS should be used to obtain estimates for the adjusted differences between treatment means and the error associated with these differences.

3. The LSMEANS statement should be used to calculate least-square means for treatments.

4. The two one-sided tests procedure (12) should be used to calculate 90% confidence intervals for the mean difference for AUC and C_{max}, which should generally be within $\pm$ 20% of the corresponding reference mean.

Adverse Reactions: The sponsor should report all adverse reactions that occurred during the study with regard to the nature, onset, duration, frequency, severity, type of treatment during which the reaction occurred and the suspected relation to the drug treatment.

III. *IN VITRO* TESTING

A. Dissolution Testing

The sponsor should conduct dissolution testing on 12 individual dosage units of both the test and reference products using USP XXII apparatus I (basket) at 50 rpm in 750 ml of 0.1 N HCl at 37 ^{o} C. Not less than 80% of the labeled amounts of both drugs should be dissolved in 30 minutes.

For the dissolution testing, the sponsor should include the following data:

1. Comparative dissolution for the test and reference products at 10, 20, 30, and 45 minutes.

2. For each time interval, the percent dissolution for each dosage unit being tested.

3. For each time interval, the mean percent dissolved, the range of percent dissolution for the 12 units, and the coefficient of variation.

4. The validated analytical method used.

5. Lot numbers for both test and reference products. These must be the same lots that were used in the bioequivalence study.

6. Expiration date for the reference product.

B. Content Uniformity Test

The sponsor should submit content uniformity data for

all the test and reference lots used in the dissolution testing.

C. Potency

The sponsor should submit potency data for all the test and reference lots used in the dissolution testing.

IV. WAIVER REQUESTS

The sponsor may submit waiver requests for the 10 mg/100 mg and 25 mg/100 mg tablets of carbidopa and levodopa which should include the following:

A. A side-by-side comparison of the composition (names and quantities of active and inactive ingredients) of the 25 mg/250 mg and lower strength tablets.

B. Dissolution data for the lower strength tablets as described in Section III.

V. REFERENCES

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